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Novel Compositions

This invention relates to novel vaccine formulations, methods for preparing them and their use in therapy. In particular the present invention relates to combination vaccines for administration to children and the elderly.

Streptococcus pneumoniae is a gram positive bacteria responsible for considerable morbidity and mortality, particularly in the young and aged. Expansive colonisation of the respiratory tract, and middle ear, especially in young children, is the single most common cause for hospital visits in the US. The bacteria may become invasive, infecting the lower lungs and causing pneumonia. The rate of pneumococcal pneumonia in the US for persons over 60 years of age is estimated to be 3 to 8 per 100,000. In 20% of cases this leads to bacteremia, and other manifestations such as meningitis, with a mortality rate close to 30% even with antibiotic treatment. There are 90 known serotypes of *Streptococcus pneumoniae* which are determined by the structures of the capsular polysaccharide surrounding the bacteria, and this is its major virulence factor.

A 17 - valent pneumococcal vaccine (Moniarix) is known, based on the purified polysaccharides of the pneumococcal serotypes most commonly involved in invasive disease. The method of purification of these polysaccharides was disclosed in European Patent 72513 B1. Vaccine efficacy trials with lower valent vaccines demonstrated a 70 to 90% efficacy with respect to serotypes present in the combination. Case controlled studies in the US in persons >55 years using a 14 valent vaccine demonstrated 70% efficacy (Mills, O.F., and Rhoads, G.G; J. Clin. Epidemiol. (1996); Vol.49(6) 631-636). Inclusion of additional polysaccharides (to make a 23-valent pneumococcal vaccine) were accepted on the basis of an adequate serological response, even though there was clinical efficacy data lacking (K. R. Brown In 'Combined.Vaccines and Simultaneous Administration', Ed. Williams et al. New York Academy of Sciences 1995 pp 241-249).

Simultaneous immunisation with a 23-valent pneumococcal vaccine (Pnu-Immune 23 - Lederle USA) and influenza vaccine (Fluarix) has been studied (TJ Fletcher, TW Tunnicliffe, K Hammond, K Roberts, JG Ayres; (1997) Br. Med. J. 314: 1663) and no significant immunological differences were noted between simultaneous immunisation, or immunisations one month apart.

Pneumococcal polysaccharides can be rendered more immunogenic in infants by chemically coupling them to protein carriers, and clinical efficacy trials are being performed to verify this concept for efficacy in preventing infant Otitis media.

Infants primed with a 7 to 11-valent conjugate pneumococcal vaccine may be boosted with the 23-valent plain polysaccharide pneumococcal vaccine in order to increase the serotype coverage and IgG concentrations (Block, SL, JA. Hedrick, R.A. Smith, R.D. Tyler, M. Giordani, M.D. Blum, J. Sadoff, E. Keegan; Abstract G-88, 37th ICAAC, Toronto (1997); Anderson, E.L., Kennedy, D.J., Geldmacher, K.M., Donnelly, J., Mendelman, P; The Journal of Paediatrics, May 1996. Vo. 128, n°5, Part 1, 649-653).

Respiratory Syncytial virus (RSV) occurs in seasonal outbreaks, peaking during the winter in temperate climates and during the rainy season in warmer climates (DeSilva LM & Hanlon MG. Respiratory Syncytial Virus: a report of a 5-year study at a children's hospital. *J Med Virol* 1986;**19**:299-305). Wherever the area, RSV tends to have a regular and predictable pattern and other respiratory viral pathogens that occur in outbreaks are rarely present concurrently (Glezen WP & Denny FW. Epidemiology of acute lower respiratory disease in children. *N. Engl. J. Med.* 1973;**288**:498-505).

RSV infection is almost certainly underdiagnosed in adults, in part because it is considered to be an infection of children. Consequently, evidence of the virus in adults is not sought in order to explain respiratory illness. In addition, RSV is difficult to identify in nasal secretions from individuals who have some degree of partial immunity to the virus, as do the large majority of adults. Young to middle-age adults typically develop a persistent cold-like syndrome when infected with RSV. Elderly individuals may develop a prolonged

respiratory syndrome which is virtually indistinguishable from influenza, with upper respiratory symptoms which may be accompanied by lower respiratory tract involvement, including pneumonia. Institutionalised elderly populations are of particular concern, because they comprise large numbers of susceptible individuals clustered together. The spread of infection through such a population, many of whom have multiple medical problems which may predispose them to a more severe course of the disease, is difficult to control.

Furthermore, reports of recent studies evaluating the impact of RSV infection as a cause of hospitalisation in adults and in community dwelling healthy elderly further point to an important role of RSV infection in severe lower respiratory tract disease in these populations (Dowell SF, Anderson LJ, & Gary Jr HE, *et al. J. Infect Dis* 1996;**174**:456-462; Falsey AR, Cunningham CK, & Barker WH, *et al. J. Infect Dis* 1995;**172**:389-394). Dowell identified RSV as one of the four most common pathogens causing severe lower respiratory tract disease resulting in hospitalisation of adults (Dowell SF, Anderson LJ, & Gary Jr HE, *et al. J. Infect Dis* 1996;**174**:456-462). Falsey demonstrated that serious RSV infections in elderly persons are not limited to nursing homes or outbreak situations. Rather, RSV infection is a predictable cause of serious illness among elderly patients residing in the community. Similar to hospitalisations for influenza A, those related to RSV infections were associated with substantial morbidity, as evidenced by prolonged hospital stays, high intensive care admission rates, and high ventilatory support rates (Falsey AR, Cunningham CK, & Barker WH, *et al. J. Infect Dis* 1995;**172**:389-394).

These studies point to the medical and economic need for an effective vaccine which can prevent severe complications of RSV infection in infants, adults and both community dwelling healthy and institutionalised elderly.

SmithKline Beecham has undertaken the development of an RSV vaccine based on the extracellular domain of the F and G surface glycoproteins of RSV strain A. The use of an adjuvant is of primary importance for a subunit recombinant vaccine. Alum is the only adjuvant currently licensed for human use. However, Alum has a limited adjuvant effect

on the humoral response, and is known to induce mainly a TH2 cellular response (Byars NE, Nakano G, & Welch M, *et al. Vaccine* 1991;**9**:309-318). It has been found, however, that 3 De-O-acylated monophosphoryl lipid A (3D-MPL) in combination with Alum improves the humoral response and stimulates preferentially a TH1-type immunity. The Aluminium salt with 3D-MPL adjuvant is denoted Alum/3D-MPL.

The present invention provides a vaccine composition comprising:

(a) one or more *Streptococcus pneumoniae* polysaccharides either conjugated to a protein or peptide, or non-conjugated; and
(b) an RSV antigen
in combination with an adjuvant which is a preferential stimulator of TH1 cell response.

The trend towards combination vaccines has the advantage of reducing discomfort to the recipient, facilitating scheduling, and ensuring completion of regiment; but there is also the concomitant risk of reducing the vaccine's efficacy. It would be, therefore, advantageous to make vaccine combinations which meet the needs of a population, and which, in addition, do not interfere with each other. It would be of further advantage if the combination of the vaccines results in synergy with resulting improvement of one or both vaccines efficacy, or improved correlates of protections for one or both vaccines. This is achieved by the vaccine composition of the invention which is of great benefit for administration to children or the elderly who may be particularly at risk of *Streptococcus pneumoniae*, and/or RSV infection.

Optionally the vaccine composition of the invention additionally comprises one or more of a number of other antigens such as an antigen against *influenza* virus. Currently available influenza vaccines include whole inactivated virus vaccines, split particle vaccines, and subunit vaccines. Inactivated influenza vaccines, of all kinds, are usually trivalent vaccines. They contain antigens derived from two influenza A virus strains and one

influenza B strain. A standard 0.5ml dose of most of them contain 15μg of haemagglutinin component from each strain.

The procedure for determining the strains to be incorporated into an influenza vaccine is a complex process which involves collaboration between the World Health Organisation, national health authorities and vaccine manufacturers.

There are two conjugation methods generally used for producing immunogenic polysaccharide constructs: (1) direct conjugation of carbohydrate and protein; and (2) indirect conjugation of carbohydrates and protein via a bifunctional linker or spacer reagent. Generally, both direct and indirect conjugation require chemical activation of the carbohydrate moiety prior to derivatisation. See for example US 5,651,971 and Dick & Beurret, "Glycoconjugates of Bacterial Carbohydrate Antigens," Conjugate Vaccines, J.M. Cruse & R.E. Lewis (eds), Vol. 10, 48 - 114 (1989).

The *Streptococcus pneumoniae* polysaccharides, if conjugated, are conjugated to either a protein or a peptide. Polysaccharide antigens have been conjugated to a number of T helper proteins. These provide T-helper epitopes. Representative proteins include Diphtheria Toxoid, Tetanus toxoid, and protein D or its lipidated derivative lipoprotein D from Heamophilius influenzae B. Other suitable protein carriers include, Diphtheria Crm 197 and the major non structural protein from influenzae, NS1 (particularly amino acid 1-81).

The *Streptococcus pneumoniae* polysaccharide in the composition of the invention is preferably a vaccine with a number of valencies, preferably at least an 11-valent vaccine, for example a 17- or 23-valent vaccine. Most preferably the 23-valent pneumococcal polysaccharide or the 11-valent conjugate vaccine.

Suitable RSV antigens for inclusion in vaccines include an inactivated RSV virus, such as a formalin inactivated RSV virus, or antigens derived from the RSV virus, preferably human RSV envelope glycoproteins, such as the RSV F or G protein or immunogenic fragments

thereof as disclosed for example in US Patent 5149650, or a chimeric polypeptide comprising at least one immunogenic fragment from both RSV F and G proteins, advantageously an RSV FG chimeric protein as disclosed for example in US patent 5194 595 preferably expressed from CHO cells.

The RSV antigen in the composition of the invention is preferably an RSV FG chimeric protein.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in International Patent Application No. 92/116556.

Preferably, the size of the particles of 3D-MPL are small enough to be sterile filtered through a 0.22micron membrane (as described in European Patent number 0689454).

3D-MPL will be present in the range of 10μg - 100μg preferably 25-50μg per dose wherein the antigen will typically be present in a range 2-50μg per dose.

Another preferred adjuvant comprises QS21, an Hplc purified non-toxic fraction derived from the bark of Quillaja Saponaria Molina. Optionally this may be admixed with 3 De-O-acylated monophosphoryl lipid A (3D-MPL), optionally together with an carrier.

QS21 is a Hplc purified non-toxic fraction of a saponin from the bark of the South American tree Quillaja Saponaria Molina and its method of its production is disclosed (as QS21) in US patent No. 5,057,540.

Non-reactogenic adjuvant formulations containing QS21 have been described previously (WO 96/33739). Such formulations comprising QS21 and cholesterol have been shown to be successful TH1 stimulating adjuvants when formulated together with an antigen. Thus vaccine compositions which form part of the present invention may include a combination of QS21 and cholesterol.

Further adjuvants which are preferential stimulators of TH1 cell response include immunomodulatory oligonucleotides, for example unmethylated CpG sequences as disclosed in WO 96/02555.

Combinations of different TH1 stimulating adjuvants, such as those mentioned hereinabove, are also contemplated as providing an adjuvant which is a preferential stimulator of TH1 cell response. For example, QS21 can be formulated together with 3D-MPL. The ratio of QS21 : 3D-MPL will typically be in the order of 1 : 10 to 10 : 1; preferably 1:5 to 5 : 1 and often substantially 1 : 1. The preferred range for optimal synergy is 2.5 : 1 to 1 : 1 3D MPL: QS21.

Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or alum.

A preferred oil-in-water emulsion comprises a metabolisible oil, such as squalene, alpha tocopherol and tween 80. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

In a preferred aspect aluminium hydroxide (alum) or aluminium phosphate will be added to the composition of the invention to enhance immunogenicity.

In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with 3D-MPL and alum.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1 μg - 200 μ g, such as 10-100 μ g, preferably 10 μ g - 50 μ g per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal or less than 1 as this provides amore stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The vaccine of the present invention will contain an immunoprotective quantity of the antigens and may be prepared by conventional techniques.

Vaccine preparation is generally described in Pharmaceutical Biotechnology, Vol.61 Vaccine Design - the subunit and adjuvant approach, edited by Powell and Newman, Plenum Press, 1995; New Trends and Developments in Vaccines, edited by Voller et al., University Park Press, Baltimore, Maryland, U.S.A. 1978. Encapsulation within liposomes is described, for example, by Fullerton, U.S. Patent 4,235,877. Conjugation of proteins to macromolecules is disclosed, for example, by Likhite, U.S. Patent 4,372,945 and by Armor et al., U.S. Patent 4,474,757.

The amount of protein in each vaccine dose is selected as an amount which induces an immunoprotective response without significant, adverse side effects in typical vaccinees. Such amount will vary depending upon which specific immunogen is employed. Generally, it is expected that each dose will comprise 1-1000 mg of protein, preferably 2-100 mg, most preferably 4-40 mg. An optimal amount for a particular vaccine can be

ascertained by standard studies involving observation of antibody titres and other responses in subjects. Following an initial vaccination, subjects may receive a boost in about 4 weeks.

In addition to vaccination of persons susceptible to *Streptococcus pneumoniae* or RSV infections, the pharmaceutical compositions of the present invention may be used to treat, immunotherapeutically, patients suffering from the said infections.

In a further aspect of the present invention there is provided a method of manufacture as herein described, wherein the method comprises mixing a *Streptococcous pneumoniae* antigen and an RSV antigen with a Th-1 inducing adjuvant, for example 3D-MPL and, preferably, a carrier, for example alum.

If desired, other antigens may be added, in any convenient order, to provide multivalent vaccine compositions as described herein.

In the examples that follow, evaluation of the effect of combining the RSV vaccine candidate, FG with the commercially available 23-valent pneumococcal vaccine (Pneumune from Wyeth-Lederle) in a mouse model was carried out. This model tested a combined immunisation where the vaccines are physically mixed and injected in the same site. The results of this study demonstrate a mutual synergy, resulting in improved responses for certain immunological markers for both vaccines.

EXAMPLES

1. Formulation process:

H_2O-diluted FG antigen (2μg:1/10 HD) was adsorbed for 15 min on 50 μg of $Al(OH)_3$. When used, 5μg of 3D-MPL were added to the preparation as a suspension of 100 nm particles and incubated for 30 min. Formulations were then buffered with 10-fold concentrated PBS pH 7.4. When used, the 23 valent pneumococcal vaccine (11.5μg:1/50 HD) was added 15 min. after the addition of the buffer solution. For groups without FG the same formulation sequence was followed so that the 3D-MPL was first adsorbed on $Al(OH)_3$ before adding the 23 valent pneumococcal vaccine. Fifteen minutes after the addition of the concentrated buffer or the 23 valent pneumococcal vaccine phenoxyethanol (5mg/ml) was added to the formulations as preservative.

All incubations were carried out at room temperature with agitation. The formulations were prepared simultaneously for the 2 injections with a 7-day maturation of the finalised formulations before the first injection.

Composition of formulation constituents:

COMPONENT	BATCH NUMBER	CONCENTRATION (μg/ml)	BUFFER
FG	54/023	265	10 PO4, 150NaCl pH6.8
$Al(OH)_3$	96A0089	10380	H_2O
23-Valent	Pneumune	1150	saline
3D-MPL	109	964	H_2O

2. Immunization protocol:

11 groups of 10 mice were immunised by different routes (50 μl) at days 0 and 28 with various formulations (see ***Table 1***). Group 7 and 8 were immunised with live RSV by the intra-nasal route (60μl). Sera were obtained at days 28 (28 d Post I) and 42 (14 d Post II). On day 42, spleen and node cells were taken from 5 mice of groups 4, 5, 6, 7, 8, 9, as well as from 5 naïve Balb/c mice (group 1, not immunised).

3. Humoral response.

3.1. Anti-FG antibodies:

All humoral results were performed for 10 mice/group (individual response for the anti-FG titers and pooled sera for the isotype profile) and cellular results were presented for 5 mice/group.

Individual sera were obtained 28 days after the first Immunization and 14 days after the second immunisation and were tested for the presence of FG specific Ig antibodies and their isotype (IgG2a, IgG1) distribution.
The assay protocol was as follows: coating overnight at 4°C with 50 µl of purified FG 54/023 (1µg/ml) per well, saturation 1h at 37°C, incubation with sera 1h30 at 37°C, incubation with anti-mouse Ig biotin 1/1500 (or IgG1, IgG2a biotin 1/1000) 1h30 at 37°C, incubation with strepta-peroxydase 1/2500 30 min at 37°C, incubation with OPDA Sigma 15 min at RT, stop with H_2SO_4 2N.
OD were monitored at 490 nm and the titers determined by linear regression (y=a.logx + b): titer = serum dilution giving 50% reduction of the maximal OD. 95% confidence limits (IC 95%) were calculated for each group.

Individual sera obtained 14d Post II were tested for the presence of neutralising antibodies using the following protocol: 50 µl of serial two-fold dilutions of sera (first dilution 1/250) were incubated for 1 hour at 37°C with 50 µl of a mixture containing 500 pfu of RSV-A/Long (Lot 14) and guinea pig complement in a 96 well plate in duplicate. 100 µl of a HEp-2 cell suspension at 10^5 cells/ml were then added to each well and the plates were incubated for 4 days at 37°C in the presence of 5% CO_2.
The supernatants were then aspirated, and after addition of a 100 µl of a WST-1 preparation (dilution 1/12.5) the plates were further incubated for 24H at 37°C in the presence of 5% CO_2. The OD were monitored at 595 nm and the titers determined by linear regression (y=a.logx + b): titer = serum dilution giving 50% reduction of the maximal OD observed for the uninfected cells.
Controls in test included a pool of randomly chosen human sera (Human pool) and Sandoglobuline (lot 069, generic human IgG produced by Sandoz).

3.2. Anti-Pneumococcal Polysaccharide IgG:

Murine IgG to pneumococcal polysaccharides types 6B, 14, 19F and 23F was measured by ELISA in a method adapted from the CDC protocol. This protocol includes the addition of soluble cell wall polysaccharide (CPS) to the sera to inhibit the measurement of CPS antibodies. CPS is a phosphoryl choline containing teichoic acid common to all pneumococci. It is present under the capsule, and antibodies to it are only weakly

protective. Since CPS is linked to the capsular polysaccharide, there is usually 0.5 to 1% CPS contaminating the purified capsular polysaccharide used to coat the ELISA plates. Thus, measurement of the CPS antibodies can confound the interpretation ELISA results with respect to the capsular polysaccharide.
The ELISA was performed with polysaccharides coated at 20, 5, 10 and 20 μg/ml in carbonate buffer for types 6B, 14, 19F and 23F respectively. Sera was pre-mixed with the equivalent of 500 μg/ml CPS in undiluted sera, and incubated for 30 minutes before addition to the ELISA plate. Murine IgG was detected with Jackson ImmunoLab goat anti-murine IgG (H+L) peroxidase at 1:2000 dilution. The titration curves were referenced to polysaccharide specific murine monoclonal antibodies of known concentration for each serotype using logistic log comparison by SoftMax Pro. The monoclonals used were HASP4, PS14/4, PS19/5 and PS23/22 for types 6B, 14, 19F and 23F respectively. Due to the limited quantity of sera available, pooled sera was tested, thus statistical analysis is not available.

4. Cellular response

Spleen and lymph node cells were isolated 14d Post II from groups 4-9 and from naïve mice (Group 1) for use as a negative control for the FG-specific cellular response analysis. Samples were analysed for both FG-specific lymphoproliferation and cytokine (IFN-γ + IL-5) secretion.
Proliferation was evaluated after a 96h incubation of $4x10^5$ cells/well of 96 well plates with 200 μl of media containing 10 to 0.03 μg/ml of FG (3-fold dilutions). Upon ^{3}H-thymidine incorporation, the FG specific proliferation was measured following our standard protocol. Cytokine induction was evaluated after 96 h incubation of $2.5x10^6$ cells per well of 24 well with 1 ml of media containing 10μg to 0.01μg of FG (10-fold dilutions). Supernatants were then harvested to determine the quantity of IFN-γ and IL-5 induced by ELISA following our standard protocol.

Results

1. GROUPS.

10 groups received two immunisations of various formulations containing either the 23-valent vaccine or FG or a combination of both formulated with Aluminium hydroxide and 3D-MPL or Aluminium hydroxide. Group 1 constitutes the control for the CMI studies. Groups 2 and 9 will allow the evaluation of the immunogenicity of the 23 Valent pneumoccocal vaccine upon intraperitoneal immunization which was used in the literature for the evaluation of 23 Valent pneumoccocal vaccine, and upon IM immunization, Groups 3 and 10 allow a parallel analysis to groups 2 and 9 except that the 23 Valent pneumoccocal vaccine is formulated with Alum/3D-MPL. Groups 2 and 3 also constitute

controls for the impact of Alum/3D-MPL on the 23 Valent pneumoccocal vaccine when it is not combined to FG Alum/3D-MPL. Group 4 will allow the evaluation of the immune response induced upon IM Immunization of the combination of the 23 Valent pneumoccocal vaccine and FG Alum/3D-MPL. Groups 5 and 6 constitute a control for the evaluation of the impact of Alum/3D-MPL on FG when it is not combined with the 23 Valent pneumoccocal vaccine. The RSV live immunisations was a control for the immune response induced upon natural RSV IN infection (Groups 7). Finally, group 9 is a control for the impact of Alum/3D-MPL alone.

TABLE 1

Groups	Antigen	Adjuvant	Route
1	none	none	
2	23 Valent Pneumune(11.5μg)	none	IM
3	23 Valent Pneumune (11.5μg)	Alum/3D-MPL	IM
4	23 Valent Pneumune (11.5μg) / FG (2μg)	Alum/3D-MPL	IM
5	FG (2μg)	Alum/3D-MPL	IM
6	FG (2μg)	Alum	IM
7	RSV Live (Lot 14: 10^5PFU)	none	IN
8	none	Alum/3D-MPL	IM
9	23 Valent Pneumune (11.5μg)	none	IP
10	23 Valent Pneumune (11.5μg)	Alum/3D-MPL	IP

2. HUMORAL RESPONSE.

2.1. Anti-FG antibodies

- The analysis of specific anti-FG antibodies at 28 d Post I and 14 d Post II shows the induction of similar antibody responses upon Immunization with FG Alum/3D-MPL / 23-Valent (Group 4) or FG Alum/3D-MPL alone (Group 5) with slightly higher titers observed for FG Alum/3D-MPL / 23-Valent (***Figure 1***). The Immunization with FG Alum (Group 6) induces expectedly lower antibody titers at both time points. Statistical analysis shows that there is indeed a significant difference between the anti-FG Ig titers induced by Groups 4 -6 at 28 d Post I and 14 d Post II.

RESULTS

- The analysis of anti-RSVA neutralizing antibodies (***Figure 2***) presents a parallel profile as the one observed for the anti-FG Ig responses.

- Based on the above results, the anti-FG / anti RSV neutralizing antibody ratio (28d PostII) was calculated and showed that the ratio of FG Alum/3D-MPL / 23-Valent, FG Alum/3D-MPL and FG Alum compares to the ratio induced by the RSV IN group (***Figure 3***).

- An in depth statistical analysis on individual anti-FG Ig, IgG1 and IgG2a isotype titers showed that the addition of the 23-valent vaccine to FG Alum/3D-MPL maintains the Ig and IgG1 responses and increases significantly the IgG2a responses (***Figure 4A***). In addition, the analysis showed that the IgG1/IgG2a ratio and the IgG1 titer of FG Alum/3D-MPL / 23 valent and FG Alum/3D-MPL were similar although significantly different from FG Alum (***Figure 4B***). The three formulations had significant differences in their IgG2a titers.

2.2. Anti-Pneumococcal Polysaccharide IgG

While mice and other rodents may produce IgM against polysaccharide immunogens, they do not normally produce IgG against polysaccharides. This is because their immune system lacks the signals to induce isotype switching to a T-independent antigen such as a polysaccharide. Thus, the measurement of anti-polysaccharide IgG in mice is a sensitive test for the induction of an improved T-independent immune response.

The concentrations of IgG induced against four of the 23 polysaccharides are presented in ***Figure 5***. As we had noted in other experiments, no IgG was produced against polysaccharide types 6B and 23F. However, there was measurable IgG produced against types 14 and 19F.

- Groups that did not contain 23-Valent vaccine did show any detectable IgG to polysaccharides 14 and 19F, confirming the specificity of the measurements.

- A comparison of the route of immunisation reveals that when the 23-valent vaccine is adjuvanted with Alum/3D-MPL, the intra-muscular route appears to be better than inter-peritoneal for type 19F, whereas the reverse is true for type 14. With plain 23-Valent, there does not seem to be a significant difference.

- 23Valent + Alum/3D-MPL induces greater IgG for types 14 and 19F, in both IM and IP immunisation routes when compared to 23-Valent alone. When combined with FG,

however, there is a reduction in the IgG response. Nevertheless, the IgG response to 19F in 23-Valent+FG/Alum/3D-MPL is still greater than that induced by 23-Valent alone (compare groups 3 and 4), and the response to type 14 is improved.

3. CELLULAR RESPONSE.

- The induced FG-specific lymphoproliferation did not show any difference between FG Alum/3D-MPL +/- 23 Valent and FG Alum, both in spleen cells and in lymph node cells (***Figure 6***).

- The analysis of the production of IL-5 and IFN-γ suggests that the mixture of the 23 valent vaccine with FG Alum/3D-MPL does not hamper the production of IFN-γ but could increase somewhat the production of IL-5 (***Figure 7***). This observation is confirmed by a 2 to 8 fold difference in the IFN-γ / IL-5 ratio observed at two doses of FG (10 and 1 μg FG/ml) used for *in vitro* restimulation. However, the FG Alum/3D-MPL / 23-valent vaccine still induces a much higher IFN-γ / IL-5 ratio than FG Alum.

CONCLUSIONS

The analysis of the induced anti-FG Ig specific and anti RSVA neutralizing antibody titers shows that the combination of FG Alum/3D-MPL with the 23 valent pneumococcal vaccine does not hamper the induction of the response observed with FG Alum/3D-MPL alone. The quality of the response measured by the anti-FG/anti-RSVA neutralizing antibody ratio also remains unchanged and close to the ratio observed upon natural infection with RSV true the IN route.

Analysis of the induced FG specific cell mediated response suggest that the addition of the 23 valent vaccine to FG Alum/3D-MPL does not affect the induction of lymphoproliferation in both spleen cells and lymph node cells. Furthermore, the induction of a Th1 type response typically observed with FG Alum/3D-MPL is not hampered by the addition of the 23 valent as measured by in vitro cytokine production of FG specific spleen and lymphnode cells and appears to be enhanced when analysing the FG specific isotype antibody distribution. Indeed, the production of IFN-γ, marker of a Th1 response, remains unchanged upon addition of the 23 valent vaccine to FG Alum/3D-MPL while the production of Il-5, marker of a Th2 response is only slightly increased. Interestingly, the addition of the 23 valent vaccine to FG Alum/3D-MPL significantly increases the production of IgG2a antibodies, marker of a Th1 response, while it is not affecting the IgG1 (marker of a Th2 response) nor the IgG1/IgG2a response.

Thus the combination of FG Alum/3D-MPL and 23-valent vaccine does not affect the response observed with FG Alum/3D-MPL and therefore the advantages linked to the FG Alum/3D-MPL formulation versus FG Alum i.e., induction of high primary and secondary neutralizing antibody responses and a Th1 response as measured by the presence of IgG2a antibodies and the induction by high levels of IFN-γ are maintained.

Combination of 23Valent Pneumune with FG Alum/3D-MPL results in increased IgG production in 2 of 4 polysaccharides tested, but most dramatically for type 19F. 23-Valent alone with Alum/3D-MPL gave the highest IgG concentrations.

In conclusion, there is a favourable combination of 23-Valent pneumococcal vaccine with RSV FG Alum/3D-MPL in which there is a synergistic effect for both vaccines in that both vaccines show improvements in certain immunological tests, and no inhibition in any tests.

Claims

1. A vaccine composition comprising:

(a) one or more *Streptococcus pneumoniae* polysaccharides either conjugated to a protein or peptide, or non-conjugated; and

(b) an RSV antigen

in conjunction with an adjuvant which is a preferential stimulator of TH1 cell response.

2. A vaccine composition according to claim 1 in which the *Streptococcus pneumoniae* polysaccharide is conjugated preferentially to a protein from the group comprising; Protein D from Haemophilus influenzae B, a lipidated version thereof (lipoprotein D); Tetanus toxin, and Diphtheria Toxin.

3. A vaccine composition according to claim 1 or claim 2 which additionally comprises a carrier.

4. A vaccine composition according to any one of claims 1 to 3 in which the preferential stimulator of TH1-cell response is selected from the group of adjuvants comprising: 3D-MPL, 3D-MPL wherein the size of the particles of 3D-MPL is preferably about or less than 100nm, QS21, a mixture of QS21 and cholesterol, and a CpG oligonucleotide.

5. A vaccine composition according to claim 4 in which the preferential stimulator of TH1-cell response is 3D-MPL.

6. A vaccine composition according to any one of claims 1 to 5 in which the *Streptococcus pneumoniae* polysaccharide is a 23-valent pneumococcal polysaccharide vaccine.

7. A vaccine compostion according to any one of claims 1 to 5 in which the *Streptococcus pneumoniae* polysaccharide is an 11-valent conjugated pneumococcal polysaccharide vaccine.

8. A vaccine composition according to any one of claims 1 to 7 in which the RSV antigen is a human RSV envelope glycoprotein.

9. A vaccine composition according to any one of claims 1 to 8 in which the RSV antigen is chimeric FG or a fragment thereof.

10. A vaccine composition according to any one of claims 1 to 9 in which an influenza virus antigen is additionally present.

11. A vaccine composition according to any one of claims 1 to 10 in which the carrier is selected from the group comprising aluminium hydroxide, aluminium phosphate and tocopherol and an oil in water emulsion.